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14. ABSTRACT

This proposal studies the role of NF1 in mediating long-term memory retrieval in Drosophila. Over the last funding period, we devoted our efforts in two areas as (1) to determine the role of NF1 in retrieval of LTM and (2) to map neural circuits involved in aversive and appetitive memory retrieval. We finally were able to overcome odds to confirm the preliminary observations that suggest a role for NF1 in retrieval of LTM. Moreover, we found that NF1 not only was involved in aversive LTM as demonstrated before but also in appetitive LTM. Appetitive LTM provides a much better behavioral paradigm for addressing the role of NF1 in mediating LTM retrieval, as NF1 specifically disrupts appetitive LTM without affecting short-term appetitive memory, while both are impaired in aversive memories. We also found that overexpression of the NF1 gene enhanced both aversive and appetitive LTM. This observation is highly significant for it allows much better accessibility to genetic manipulation in mapping neural circuits underlying retrieval of LTM.

15. SUBJECT TERMS
NF1, Drosophila, memory, retrieval, mushroom-body

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Introduction

We have reported that NF1 affects both immediate memory and long-term memory (LTM), but through different mechanisms in Drosophila (Ho et al., 2007). The goal of this proposal is of studying the hypothesis as to that in long-term memory (LTM), NF1 is specifically involved in mediating memory retrieval, but not required for induction and consolidation. For this purpose, three specific aims are proposed, including (1) to determine NF1's role in memory retrieval; (2) to identify ligands that activate NF1 for memory retrieval; and (3) to locate the brain region at which NF1-dependent memory retrieval occurs.

Body

During the first funding year of this grant (2010-2011), we encountered difficult in replicate memory phenotypes reported in the preliminary observations of this proposal, largely due to personnel changes—departure of senor graduate student and taking over the project by a freshman graduate student who had significant gap in time with the senior student. The newly involved graduate student was capable of showing mutant defects in learning and memory, but was unable to rescue such phenotype with expression of transgenes (reported in the last progress report).

Over the second funding period (2011-2012), we were continuing to make efforts in verifying the NF1's role in memory retrieval. After extensive efforts in verifying the genetic background and reestablish the appropriate genotypes, this problem was finally resolved to some extends. The student was able to show that the NF1P2 learning and memory defect, in aversive olfactory learning paradigm, was rescued through elav-Gal4 (pan-neuronal driver) driven expression of the NF1 transgene. In addition, we also made efforts in mapping neural circuits that are important for retrieval of 3-hour memory, but not long-term memory, which might provide insights for us to attack the neural circuits underlying long-term memory retrieval.
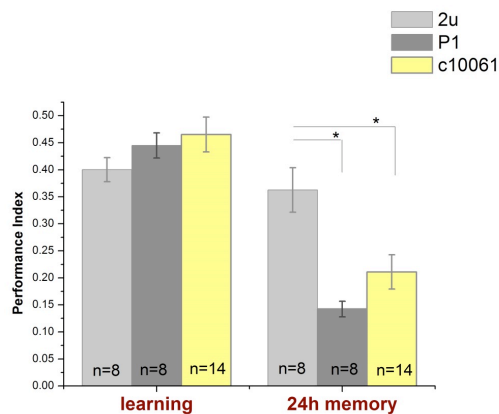

Fig. 1: Defective 24h appetitive memory in nf1 mutants. Note that learning are normal in both NF1 mutant alleles (P1 and c10061) while 24hr memory is impaired. Number of data in averages are as indicated. (*p<0.01).

Over last funding period (2012-2013), the maturation of graduate student and newly joint postdoctoral fellow allowed us making significant progress in specific aim 1 and 3. First, we showed that not only aversive LTM but also appetitive LTM was defective in insertion-induced NF1 mutants P1 and C10061 (Fig. 1) as well as in NF1 point mutations (Fig. 2). More

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importantly, appetitive learning and short-term memory were normal in all NF1 mutant alleles while only LTM was specifically impaired. The impaired appetitive LTM could be rescued by pan-neuronal expression of UAS-NF1 transgene driven by elav-Gal4 in the mutant background (elav-Gal4/+; UAS-hNF1; NF1P2) (Fig. 3). Thus, appetitive LTM provides a better behavioral paradigm for addressing the role of NF1 in retrieval of LTM.
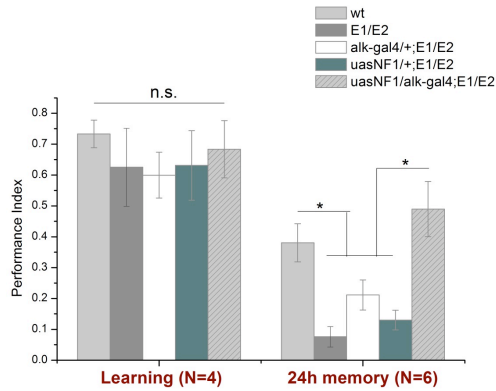
Fig. 2: Alk(38)-Gal4 expression of UAS-Nf1 in Nf1E1/E2 point mutants rescues 24h memory. For excluding effects of accumulated unknown genetic factors, heteroallelic Nf1E1/E2 mutant flies were used. E1/E2 exhibits significant 24h memory defects (*p<0.01) from parental lines, and Alk/uas-NF1; E1/E2 restores 24h memory performance.

We then showed that overexpression of NF1 at a wild-type background enhanced both aversive (not showing) and appetitive (Fig. 4) LTM. This phenotype allows better genetic accessibility than the LTM defect phenotype in the mutants for mapping neural circuits that mediate LTM retrieval (specific aim 3). We are now conducting screening of various Gal4-driven overexpression of UAS-NF1 transgene, via this enhanced LTM phenotype, to determine the brain regions that mediate NF1-dependent LTM enhancement and the role of such brain structures in retrieval of LTM.
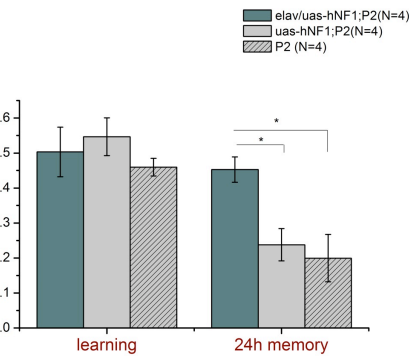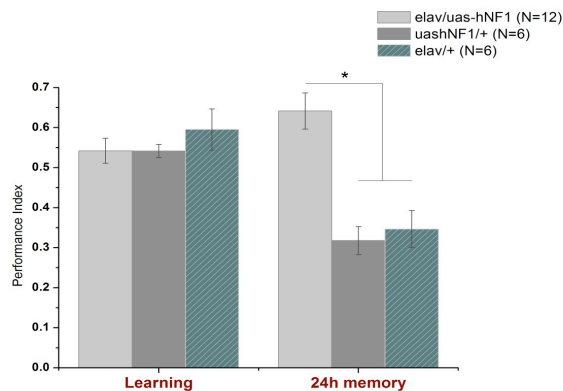

Fig. 3: Rescue of 24h appetitive memory by expressing hNF1 in nf1 null mutants. Expression of the NF1 transgene is driven by pa-neuronal driver ela (elav/+/Y;UAS-hNF1/+;Nf1P2). P1 and P2 are independent NF1 mutant alleles. N=4 for each genotype. (*p<0.01).

Fig. 4; overexpression of hNf1 enhances 24h appetitive memory. Flies with pan-neuronal overexpression of hNF1 (elav/+;hNF1 and elav/+;MBgal80/hNF1) show significant higher 24h memory performance (P<0.01). n as indicated.

Key Research Accomplishments

1. We showed that NF1 not only impairs aversive LTM but also appetitive LTM. Appetitive LTM is more suitable for studying the role of NF1 in retrieval of LTM because appetitive learning and short-term memory are normal in NF1 mutant alleles.
2. We showed that pan-neuronal overexpression of NF1 in normal transgenic flies enhances both aversive and appetitive LTM. This finding provides a much accessible way for mapping neural circuits that mediate retrieval of LTM.

Reportable Outcomes

Xie, Z., Huang, C., Cia, B., Wang, L., Zhong, Y., (in press) Requirement of the combination of mushroom body γ lobe and α/β lobes for the retrieval of both aversive and appetitive early memories in *Drosophila*. Learning and Memory.

Conclusion

(1) Through an extended period of struggling, we finally are able not only to confirm the early-observed phenotypes but also gain much stronger evidence in support a role for NF1 in retrieval of LTM specifically.
(2) We are now in a much better position examine proposed specific aims, particular number 3 specific aim for mapping neural circuits underlying LTM retrieval.

References

Ho IS, Hannan F, Guo HF, Hakker I, Zhong Y. (2007) Distinct functional domains of neurofibromatosis type 1 regulate immediate versus long-term memory formation. J Neurosci. 27, 6852-7.